

AMENDMENTS TO THE CLAIMS

1. (Currently Amended) A method for ~~preparing a~~ expanding cytotoxic lymphocyte lymphocytes which comprises:

~~expanding a cytotoxic lymphocyte~~ culturing peripheral blood mononuclear cells, Natural Killer (NK) cells, umbilical cord blood mononuclear cells, hematopoietic stem cells or blood components containing these cells in the presence of ~~[[a]]~~ at least one fibronectin fragment ~~or a mixture thereof~~ together with interleukin-2,

wherein the fibronectin fragment is

~~[[i]]~~ a polypeptide comprising at least one of the amino acid sequences of an amino acid sequence selected from the group consisting of SEQ ID NOS: 1 to 19, [[or]]

~~ii) a polypeptide having a substitution of one or more amino acids in the amino acid sequence of the polypeptide of i), and having a function which is equivalent to that of the polypeptide of i), wherein the substitution of one or more amino acids is a substitution within each of the groups of:~~

a) ~~glycine, alanine;~~

b) ~~valine, isoleucine, leucine;~~

e) ~~aspartic acid, glutamic acid, asparagine, glutamine;~~

d) ~~serine, threonine;~~

e) ~~lysine, arginine; and~~

f) ~~phenylalanine, tyrosine~~

wherein said culturing is performed for 2-15 days.

2. (Currently Amended) The method according to claim 1, wherein the ~~prepared~~ expanded cytotoxic ~~lymphocyte~~ lymphocytes highly ~~expresses~~ express an interleukin-2 receptor at a higher level than ~~[[a]] cytotoxic lymphocyte-prepared~~ lymphocytes expanded in the absence of ~~[[a]]~~ at least one fibronectin fragment ~~or a mixture thereof~~.

3. (Currently Amended) The method according to claim 1, wherein the ~~prepared~~ expanded cytotoxic ~~lymphocyte-expresses~~ lymphocytes express more CD8 than ~~[[a]] cytotoxic lymphocyte-prepared~~ lymphocytes expanded in the absence of ~~[[a]]~~ at least one fibronectin fragment ~~or a mixture thereof~~.

4. (Currently Amended) The method according to any one of claims 1 to 3, wherein the ~~prepared~~ expanded cytotoxic ~~lymphocyte-maintains~~ lymphocytes maintain cytotoxic activity longer than ~~[[a]] cytotoxic lymphocyte-prepared~~ lymphocytes expanded in the absence of ~~[[a]]~~ at least one fibronectin fragment ~~or a mixture thereof~~.

5. (Currently Amended) The method according to claim 1, wherein said at least one fibronectin fragment ~~or a mixture thereof~~ is immobilized on a solid phase.

6. (Previously Presented) The method according to claim 5, wherein the solid phase is a cell culture vessel or a cell culture carrier.

7. (Previously Presented) The method according to claim 6, wherein the cell culture vessel is a petri dish, a flask or a bag, and the cell culture carrier is beads, a membrane or a slide glass.

8. (Withdrawn) The method according to claim 1, wherein expanding a cytotoxic lymphocyte is performed in a cell culture medium comprising said fibronectin fragment or a mixture thereof.

9. (Cancelled)

10. (Currently Amended) The method according to claim 1, wherein the at least one fibronectin fragment has cell adhesion activity and/or heparin binding activity.

11. (Cancelled)

12. (Currently Amended) The method according to claim 1, comprising:
expanding a cytotoxic lymphocyte in a cell culture in the presence of said at least one fibronectin fragment ~~or a mixture thereof~~,

wherein at least (a) or (b) is true:

(a) a ratio of the number of cells present at the initiation of the cell culture to a cell culture area is 1 cell/cm² to 5 × 10⁵ cells/cm²; and

- (b) a concentration of cells at the initiation of the cell culture is from 1 cell/ml to 5×10^5 cells/ml.

13. (Cancelled)

14. (Withdrawn) A cytotoxic lymphocyte obtained by the method of claim 1.

15. (Withdrawn) A medicament comprising as an effective ingredient a cytotoxic lymphocyte obtained by the method of claim 1.

16. (Withdrawn) An agent for enhancing an interleukin-2 receptor expression of a cell, characterized in that the agent comprises as an effective ingredient fibronectin, a fragment thereof or a mixture thereof.

17. (Withdrawn) The agent according to claim 16, wherein the fibronectin fragment is a polypeptide comprising at least one of the amino acid sequences represented by SEQ ID NOs: 1 to 7 of Sequence Listing, or a polypeptide having substitution, deletion, insertion or addition of one or more amino acids in the amino acid sequence of said polypeptide, wherein the polypeptide has functions equivalent to that of said polypeptide.

18. (Withdrawn) The agent according to claim 17, wherein the fibronectin fragment has cell adhesion activity and/or heparin binding activity.

19. (Withdrawn) The agent according to claim 17, wherein the fibronectin fragment is a polypeptide selected from polypeptides comprising any one of the amino acid sequences shown in SEQ ID NOs: 8 to 19 of Sequence Listing.

20. (Withdrawn) An agent for improving a ratio of CD8-positive cell in a lymphocyte, characterized in that the agent comprises as an effective ingredient fibronectin, a fragment thereof or a mixture thereof.

21. (Withdrawn) The agent according to claim 20, wherein the fibronectin fragment is a polypeptide comprising at least one of the amino acid sequences represented by SEQ ID NOs: 1 to 7 of Sequence Listing, or a polypeptide having substitution, deletion, insertion or addition of one or more amino acids in the amino acid sequence of said polypeptide, wherein the polypeptide has functions equivalent to that of said polypeptide.

22. (Withdrawn) The agent according to claim 21, wherein the fibronectin fragment has cell adhesion activity and/or heparin binding activity.

23. (Withdrawn) The agent according to claim 21, wherein the fibronectin fragment is a polypeptide selected from polypeptides comprising any one of the amino acid sequences shown in SEQ ID NOs: 8 to 19 of Sequence Listing.

24. (Withdrawn) An agent for improving or maintaining cytotoxic activity in a cytotoxic lymphocyte, characterized in that the agent comprises as an effective ingredient fibronectin, a fragment thereof or a mixture thereof.

25. (Withdrawn) The agent according to claim 24, wherein the fibronectin fragment is a polypeptide comprising at least one of the amino acid sequences represented by SEQ ID NOs: 1 to 7 of Sequence Listing, or a polypeptide having substitution, deletion, insertion or addition of one or more amino acids in the amino acid sequence of said polypeptide, wherein the polypeptide has functions equivalent to that of said polypeptide.

26. (Withdrawn) The agent according to claim 25, wherein the fibronectin fragment has cell adhesion activity and/or heparin binding activity.

27. (Withdrawn) The agent according to claim 25, wherein the fibronectin fragment is a polypeptide selected from polypeptides comprising any one of the amino acid sequences shown in SEQ ID NOs: 8 to 19 of Sequence Listing.

28. (Currently Amended) A method for increasing expression of an interleukin-2 receptor in ~~[[a]] cytotoxic lymphocyte~~ lymphocytes, which comprises:

~~expanding a cytotoxic lymphocyte~~ culturing peripheral blood mononuclear cells, Natural Killer (NK) cells, umbilical cord blood mononuclear cells, hematopoietic stem cells or blood components containing these cells in the presence of ~~[[a]]~~ at least one fibronectin fragment ~~or a~~

~~mixture thereof~~ together with interleukin-2, thereby increasing ~~expressing~~ expression of interleukin-2 receptor in a ~~cytotoxic lymphocyte~~ the cells,

wherein the fibronectin fragment is

[[i)]] a polypeptide comprising ~~at least one of the amino acid sequences of~~ an amino acid sequence selected from the group consisting of SEQ ID NOS: 1 to 19, [[or]]

ii) ~~a polypeptide having a substitution of one or more amino acids in the amino acid sequence of the polypeptide of i), and having a function which is equivalent to that of the polypeptide of i), wherein the substitution of one or more amino acids is a substitution within each of the groups of:~~

a) ~~glycine, alanine;~~

b) ~~valine, isoleucine, leucine;~~

c) ~~aspartic acid, glutamic acid, asparagine, glutamine;~~

d) ~~serine, threonine;~~

e) ~~lysine, arginine; and~~

f) ~~phenylalanine, tyrosine~~

wherein said culturing is performed for 2-15 days.

29. (Currently Amended) A method for increasing the number of CD8-positive cells in a cytotoxic lymphocyte population lymphocytes, which comprises:

expanding a cytotoxic lymphocyte culturing peripheral blood mononuclear cells, Natural Killer (NK) cells, umbilical cord blood mononuclear cells, hematopoietic stem cells or blood components containing these cells in the presence of [[a]] at least one fibronectin fragment ~~or a~~

~~mixture thereof~~ together with interleukin-2, thereby increasing the number of ~~[[CD-8]]~~ CD8-
positive cells in ~~a cytotoxic lymphocyte population~~ the cultured cells,

wherein the fibronectin fragment is

~~[[i)]]~~ a polypeptide comprising ~~at least one of the~~ an amino acid ~~sequences~~ sequence
selected from the group consisting of SEQ ID NOS: 1 to 19, [[or]]

~~ii) a polypeptide having a substitution of one or more amino acids in the amino acid
sequence of the polypeptide of i), and having a function which is equivalent to that of the
polypeptide of i), wherein the substitution of one or more amino acids is a substitution within
each of the groups of:~~

~~a) glycine, alanine;~~

~~b) valine, isoleucine, leucine;~~

~~c) aspartic acid, glutamic acid, asparagine, glutamine;~~

~~d) serine, threonine;~~

~~e) lysine, arginine; and~~

~~f) phenylalanine, tyrosine~~

wherein said culturing is performed for 2-15 days.

30. (Currently Amended) A method for improving or maintaining cytotoxic activity in
~~[[a]]~~ cytotoxic lymphocyte lymphocytes, which comprises:

~~expanding a cytotoxic lymphocyte~~ culturing peripheral blood mononuclear cells, Natural
Killer (NK) cells, umbilical cord blood mononuclear cells, hematopoietic stem cells or blood
components containing these cells in the presence of ~~[[a]]~~ at least one fibronectin fragment ~~or a~~

~~mixture thereof together with interleukin-2, thereby improving or maintaining cytotoxic activity in a cytotoxic lymphocyte the cells,~~

wherein the fibronectin fragment is

[[i]]a polypeptide comprising ~~at least one of the~~ an amino acid ~~sequences~~ sequence selected from the group consisting of SEQ ID NOS: 1 to 19, [[or]]

~~ii) a polypeptide having a substitution of one or more amino acids in the amino acid sequence of the polypeptide of i), and having a function which is equivalent to that of the polypeptide of i), wherein the substitution of one or more amino acids is a substitution within each of the groups of:~~

~~a) glycine, alanine;~~

~~b) valine, isoleucine, leucine;~~

~~e) aspartic acid, glutamic acid, asparagine, glutamine;~~

~~d) serine, threonine;~~

~~e) lysine, arginine; and~~

~~f) phenylalanine, tyrosine~~

wherein said culturing is performed for 2-15 days.

31. (Currently Amended) The method according to claim 1, further comprising transducing a foreign gene into [[a]] the cytotoxic lymphocyte lymphocytes.

32. (Original) The method according to claim 31, wherein the foreign gene is transduced using retrovirus, adenovirus, adeno-associated virus or simian virus.

33. (Currently Amended) The method according to claim 1, wherein an expansion ratio of the cytotoxic ~~lymphocyte~~ lymphocytes is high as compared to that of ~~[[the]]~~ a method for ~~preparing~~ expanding ~~[[a]]~~ cytotoxic ~~lymphocyte~~ lymphocytes in the absence of ~~[[a]]~~ at least one fibronectin fragment ~~or a mixture thereof~~.

34. (Currently Amended) The method according to claim 1, wherein expanding ~~[[a]]~~ cytotoxic ~~lymphocyte~~ lymphocytes is performed in the presence of both of said at least one fibronectin fragment ~~or mixture thereof~~ and an anti-CD3 antibody.

35. (Currently Amended) The method according to claim 1, wherein expanding ~~[[a]]~~ cytotoxic ~~lymphocyte~~ lymphocytes is performed by incubating peripheral blood mononuclear cells or umbilical cord blood mononuclear cells.

36. (Cancelled)